Research Article

Wound Healing Activity of the Ethanol Extract of the Leaves of *Myxopyrum serratulum* A.W. Hill in Rats

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ABSTRACT

Myxopyrum serratulum (Fam. *Oleaceae*) is commonly known as 'Chaturamullai' in Tamil and 'Chaturdharalata' in Sanskrit. In Ayurveda the leaves of the plant is used as astringent, acrid, sweet, thermogenic, anodyne, febrifuge and tonic. The whole plant has considerable ethnobotanical uses in head ache, asthma, cough, fever, nerves, otitis, rheumatism and wounds. In the present study, ethanol extract of the leaves of *Myxopyrum serratulum* has been investigated for its wound healing activity by excision and dead space wound models in rats. Wound healing activity has been assessed by the rate of wound contraction, tensile strength and weight of the granulation tissue formed. Significant increase in the wound contraction has been observed on the extract animals in excision wound model. In 100 mg/kg bw, 200 mg/kg bw and 400 mg/kg bw doses of the ethanolic extract of the leaves of *Myxopyrum serratulum,* significant increase in the weight of granulation tissue and increase in tensile strength have been observed in the dead space wound model.

Keywords: Dead space wound, Excision wound, *Myxopyrum serratulum*, Wound healing.

INTRODUCTION

Myxopyrum serratulum (Oleaceae) commonly known as "Chaturamullai" is a large woody climbing shrub. The leaves are astringent, acrid, sweet, thermogenic, anodyne, febrifuge and tonic. They are useful in vitiated conditions of kapha and vata, cough, asthma, rheumatism, cephalalgia, nostalgia, consumption, fever, otopathy, neuropathy and cuts and wounds.[1] Iridoid glycosides were found on the leaves of *Myxopyrum smilacifolium.*[2]

Wound care can be traced back to early civilizations and many of these treatments were based on the use of herbal remedies. Approximately one-third of all traditional medicines in use are for the treatment of wounds and skin disorder, compared to only 1-3% of modern drugs.[3] Report about medicinal plants affecting various phases of the wound healing process, such as coagulation, inflammation, fibroplasia, collagenation, epithelization and wound contraction are abundant in the scientific literature.[4-7] Still, one should keep in mind that plants have not only beneficial effects in promoting the healing process of wounds and burns or protecting the skin from fungal and bacterial infection or anti-tumor activity against skin cancer, they can be involved in different allergic, photoallergic and irritant skin reactions.[8] Many traditional remedies are based on systematic observations and methodologies and have been time- tested but for many of them, scientific evidence is lacking. There are only few prospective randomized controlled trials that have proved the clinical efficacy of these traditional wound healing agents. The present study was designed to test the *in vivo* wound healing activity of the ethanol extract of the leaves of *Myxopyrum serratulum* A.W.Hill.

MATERIALS AND METHODS

Plant materials

The plant was collected in the month of September from Trivandrum, Kerala, India and was identified by Dr. V.Chelladurai, Former Research Officer (Botany). Central Council of Research in Ayurveda and Siddha, Government Siddha Medical College, Palayamkottai, Tamilnadu, India. A voucher specimen (MSU/PHAR/HER-139) has been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli - 627 012.

Extraction of plant material

The leaves of *Myxopyrum serratulum* were dried under shade and powdered. The dried powder (500g) was successively extracted using petroleum ether (40-60°C), benzene, chloroform, ethanol and water by using a Soxhlet apparatus. The last trace of the solvent was removed under reduced pressure by rotary evaporator. The dried crude ethanol extract has been used for the study.[9]

Animals

Wistar albino rats of either sex weighing between 180 g and 200 g were selected for the acute toxicity and wound healing activity studies. The study was approved by the Institutional Ethics Committee for animal experimentation (KMCRET/PH.D/17/2012-13), KMCH college of Pharmacy, Coimbatore. The animals were stabilized for 1 week. They were maintained in standard conditions at room temperature, 60 ± 5% relative

humidity and 12 h light dark cycle. They were given standard pellet diet supplied by Hindustan Lever Co., Mumbai and water *ad libitum* throughout the course of the study.

Acute toxicity studies

Albino rats of either sex received ethanolic extract of the leaves of *Myxopyrum serratulum* starting at 2 g/kg bw orally by gavage. The animals were observed for toxic symptoms continuously for the first 4 h after dosing. Finally, the number of survivors was noted after 24 h and these animals were then maintained for further 13 days with observations made daily.[10]

For assessment of excision wound healing activity ethanol extract was formulated in ointment by using simple ointment BP as base. 2% (w/w) ointment was applied where 2 g of the ethanol extract was incorporated in 100 g of simple ointment base BP. 0.2 g of each of the extract ointment and ciphadin ointment (std) was applied once daily to treat different groups of animals, respectively.

For the assessment of wound healing activity by dead space wound model, three dose levels (100 mg/kg bw, 200 mg/kg bw and 400 mg/kg bw) were chosen in such a way that, middle dose was approximately one-tenth of the maximum dose during acute toxicity studies, and a low dose, which was 50% of the one tenth dose, and a high dose, which was twice that of one-tenth dose.

Wound-healing activity

Excision, incision and dead space wound models were used to evaluate the wound-healing activity of *Myxopyrum serratulum.*

Excision wound model

The animals were divided into three groups of six rats each (Table 1)

Group I served as control

Group II served as standard (Ciphadin) treated with ointment topically

Group III served as test treated with *Myxopyrum serratulum* ethanol extract ointment.

All animals in each group were anaesthetized by the open mask method with anaesthetic ether before wound creation. An excision wound was inflicted by cutting away a 500 mm^2 full thickness of skin from a predetermined area, the wound was left undressed to the open environment and the wound contraction and wound closure time was monitored. Wound contraction was measured as percentage contraction in each 2 days after wound formation from the healed wound, a specimen sample of tissue was isolated from each rat for histopathological examination.[11]

Dead space wound model

The animals were divided into five groups of six rats each and kept in separate cages (Table 2)

Group I served as control

Group II served as standard (Ciphadin) treated with ointment topically

Group III was treated with 100 mg/kg bw ethanolic extract of *Myxopyrum serratulum*

Group IV was treated with 200 mg/kg bw ethanolic extract of *Myxopyrum serratulum*

Group V was treated with 400 mg/kg bw ethanolic extract of *Myxopyrum serratulum*

The model was used for the study of granuloma tissue. Animals were anaesthetized by light ether and wound was made by implantation of two polypropylene tubes (2.0 x 0.5 cm) one on either side, in the lumber region on the dorsal surface of each rat. In the ninth post-wounding day, granuloma tissue formed on an implanted tube was dissected out carefully. Granuloma tissue from one tube was dried (60°C) and stored in 10% formalin for the biochemical parameters and histopathological study. While the other part of granuloma tissue was used for the determination of tensile strength.[12,13]

Wound contraction and epithelization time

An excision wound margin was traced after wound creation by using transparent paper and area measured by graph paper. Wound contraction was measured in each 2 days interval, until complete wound healing and expressed in percentage of healing wound area. The epithelization time was measured from initial day.[14]

Measurement of tensile strength

Tensile strength is the resistance to breaking under tension. It indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue. Sutures were removed on the day 9 after wound creation and the tensile strength was measured with the help of tensiometer, which is based on method of Kuwano.[15] In this method, wound breaking strength was measured as the weight of water at the time of wound breaking per area of the specimen.

Histopathological studies

Wound tissue specimens from control, test and standard groups were taken after complete healing excision and dead space wound and after usual processing. 6 mm thick sections were cut and stained with haematoxylin and eosin.[16] Sections were qualitatively assessed under the light microscope and observed in respect of fibroblast proliferation, collagen formation, angiogenesis, epithelization, and hydroxyproline estimation. Tissues were dried in a hot air oven at 60°C-70°C to constant weight and were hydrolyzed in 6 N HCl at 130ºC for 4 h in sealed tubes. The hydrolysate was neutralized to pH 7.0 and was subjected to chloramine-T oxidation for 20 min. The reaction was determinated by addition of 0.4 M perchloric acid and colour was developed with the help of Ehrlich reagent at 60°C [17] and measured at 557 nm using a spectrophotometer.

Statistical Analysis

The means of wound area measurements and epithelization period between groups was compared using a one-way ANOVA. Treated group was compared with the control group. The results were analyzed statistically using Dunnet's't'-test to identify the differences between the treated and control groups. The data were considered significant at p<0.01.

RESULTS

Wound contraction

The percentage wound contraction was determined using the following formula:-

$$\text{percentage wound contraction} = \frac{\text{Healed area}}{\text{Total wound area}} \times 100$$

Wound area was measured by tracing the wound margin using a transparent paper in each 2 days interval and healed area calculated by subtracting from the original wound area by using graph paper. On day 15, the wound contraction of standard and ethanol extract ointment treated groups was found to be significant (p<0.01) in comparison to simple ointment base treated group. On day 19, ethanol extract ointment treated wound was completely healed while standard ointment treated group was also almost at complete healing stage, and simple ointment base treated group showed 67.85% healing. It was also observed that epithelization period of treated and standard group were less in comparison to simple ointment base treated group (Table 1).

Tensile strength

Tensile strength for the treated group on day 19 was found to be significant (p<0.05) than control group as shown in Table 2.

Table 1: Effect of the ethanol extract of the leaves of *Myxopyrum serratulum* and standard ointment on percentage wound contraction and epithelialization period of excision wound model in rats.

Days	Control		Standard (Ciphadin)		Ethanol Extract	
	Wound area	Percentage of wound contraction	Wound area	Percentage of wound contraction	Wound area	Percentage of wound contraction
0day	535 ± 18.3 (0.0%)	-	508 ± 38.5 (0.0%)	-	522 ± 31.8 (0.0%)	-
3day	508 ± 21.0 (5.04%)	94.9	450 ± 34.4 (11.42%)	88.5	490 ± 28.4 (6.13%)	93.8
5day	460 ± 17.6 (14.01%)	85.9	320 ± 28.8[a] (37.00%)	62.9	398 ± 28.0(23.75%)	76.2
7day	400 ± 12.0 (25.23%)	74.7	264 ± 31.5[a] (48.03%)	51.9	290 ± 24.8[a](44.44%)	55.5
9day	369 ± 16.5 (31.02%)	68.9	186 ± 25.2[a] (63.38%)	36.6	165 ± 25.3[a] (68.39%)	31.6
11day	309 ± 14.5 (42.24%)	57.7	104 ± 22.0[b] (79.52%)	20.4	110 ± 20.7[b] (78.92%)	21.0
13day	278 ± 13.3 (48.03%)	51.9	80 ± 18.4[b] (84.25%)	15.7	76 ± 18.2[b] (85.44%)	14.5
15day	208 ± 12.7 (61.12%)	38.8	46 ± 21.8[b] (90.94%)	9.0	30 ± 10.4[b] (94.25%)	5.7
17day	172 ± 10.2 (67.85%)	32.1	8 ± 7.1[b] (98.42%)	1.5	10 ± 5.3[b] (98.08%)	1.9
19day	166 ± 12.0 (68.97%)	31.0	0.0[b] (100%)	0	0.0[b] (100%)	0
Epithelization time (days)	23		17		17	

Values are mean ± SEM (N=6); P values vs. respective control by Dunnet's t-test: [a]P<0.01, [b]P<0.001.

Table 2: Effect of the ethanol extract and standard ointment on various parameters of dead space wound model in rats.

Groups	Doses	Granuloma (mg)		Granuloma breaking strength (g)	Hydroxyproline content (µg/ml)
		Wet weight	Dry weight		
Control	-	68.1 ± 8.9	24.9 ± 3.4	256.8 ± 2.15	1.868 ± 0.03
Standard (Ciphadin)	50mg/animal	146.4 ± 4.43*	54.9 ± 3.19*	347.8 ± 3.8*	5.267 ± 0.02*
Myxopyrum serratulum ethanol extract	100mg/kg	86.6 ± 9.8*	33.8 ± 3.5*	270.7 ± 4.38*	3.364 ± 0.04*
Myxopyrum serratulum ethanol extract	200mg/kg	85.3 ± 7.1*	35.3 ± 2.7*	314.8 ± 5.3*	4.325 ± 0.04*
Myxopyrum serratulum ethanol extract	400mg/kg	88.7 ± 13.0*	36.2 ± 4.4*	338.0 ± 2.6*	4.847 ± 0.04*

Values are mean ± SEM (N=6); P values vs. respective control by Dunnet's t-test: *P<0.05.

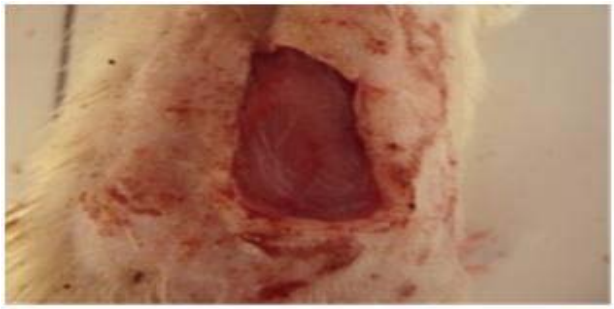

Figure 1a: Excision wound on the 0 day

Figure 1b: Wound healed treatment (control)

Figure 1c: Wound healed treatment (standard)

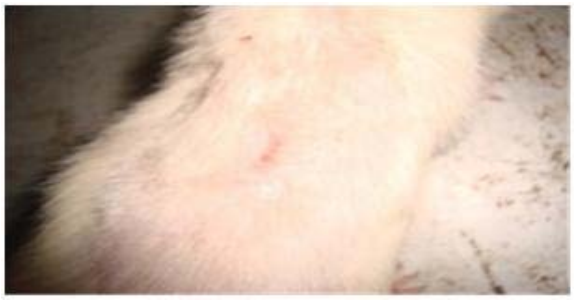

Figure 1d: Wound healed treatment (ethanol extract 2% w/w)

Figure 1: Albino rats dorsal wound area photographed at 19 days after application of the ethanol extract of *Myxopyrum serratulum* leaves by Excision model

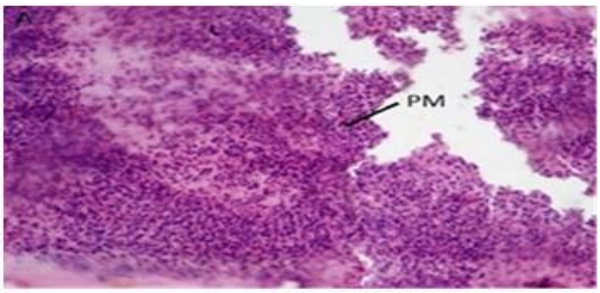

Figure 2a: Wound tissue treated with control

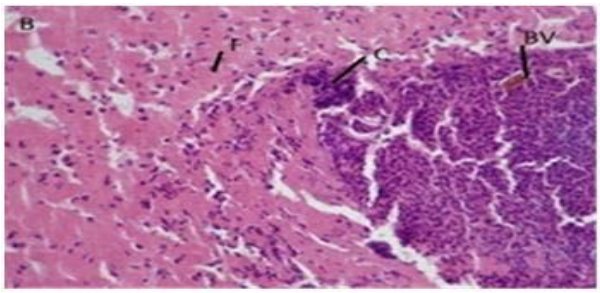

Figure 2b: Wound tissue treated with standard

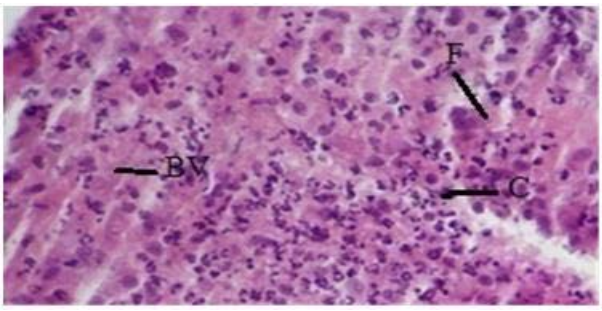

Figure 2c: Wound tissue treated with ethanol extract (2% w/w)

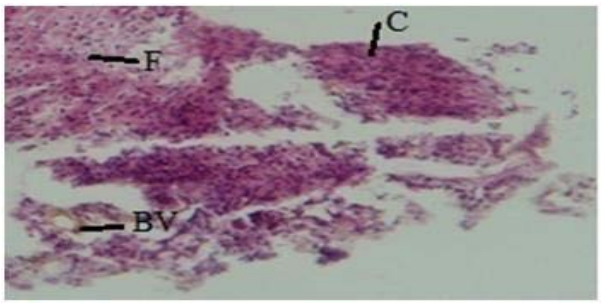

Figure 2d: Dead space model with ethanol extract (100mg/kg bw)

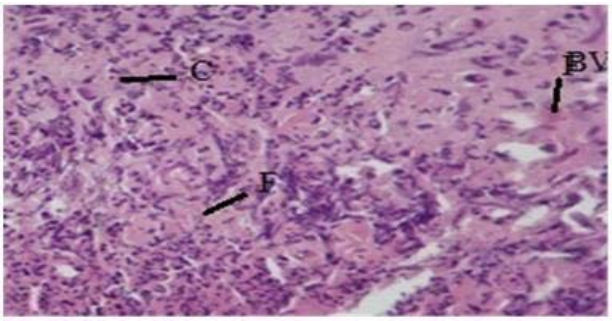

Figure 2e: Dead space model with ethanol extract (200mg/kg bw)

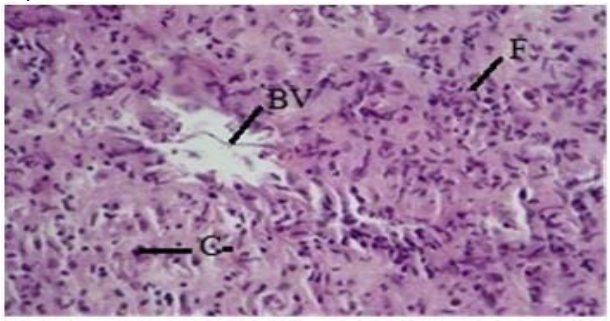

Figure 2f: Dead space model with ethanol extract (400mg/kg bw)

Figure 2: Photomicroscopic view of histopathological characteristics of 9-day-old wound tissue section by Excision and Dead space model (Hematoxylin and Eosin x 40)

Hydroxyproline and granuloma weight

Treated group showed significant increased hydroxyproline level when compared to control group ($p<0.05$) (Table 2). Granuloma weight of treated animal groups was found to be increased when compared with that of the control group.

DISCUSSION

Wound healing process consists of different phases such as granulation, collagenation, collagen maturation and scar maturation which are concurrent but independent to each other. Hence in this study two different models were used to assess the effect of ethanol extract of *Myxopyrum serratulum* on various phases.

The result showed that the ethanol extract ointment possesses a definite prohealing action. In excision wound healing model the ethanol extract of the leaves of *Myxopyrum serratulum* showed significant increase in percentage closure of excision wounds by enhanced epithelization. Significant increase, ($p<0.01$) in tensile strength, granuloma tissue and hydroxyproline content, which was further supported by histopathological studies and gain in granuloma breaking strength. This indicated improved collagen maturation by increased cross linking. An increase in dry granuloma weight indicated higher protein content.

The preliminary phytochemical analysis of the ethanolic extract of the *Myxopyrum serratulum* leaves showed the presence of flavonoids, phenolic compounds, steroidal glycosides and triterpenoids.[18] These phytochemical constituents may be responsible for the wound healing activity.

CONCLUSION

In conclusion, the study showed that the ethanol extract ointment of the leaves of *Myxopyrum serratulum* effectively stimulates wound contraction, increases tensile strength of dead space wounds as compared to control group. These findings justify the inclusion of this plant in the management of wound healing.

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